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POSSIBLE MECHANISM FOR CHANGES IN GLYCOGEN
METABOLISM IN UNLOADED SOLEUS MUSCLE



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Six days of tail-cast suspension caused marked alterations in glycogen metabolism in the soleus muscles from female rats. The *in vivo* glycogen content in the soleus of suspended rats (TCS) was significantly higher than in the soleus (SOL) from tail-casted, weight bearing controls (TCWB). In muscles depleted of glycogen by preincubation with isoproterenol, glucose metabolism in the SOL from TCS animals was found to be significantly more responsive to insulin (100 uU/ml) than the loaded SOL. Measured parameters included net glycogen turnover, glucose oxidation, and release of lactate and pyruvate. No differential effect due to insulin was observed for pyruvate oxidation or for pyruvate incorporation into glycogen. In non-glycogen-depleted muscles, the uptake of 2-deoxyglucose in the presence of insulin was much greater in the unloaded SOL than in the loaded muscle. These results suggest that the accumulation of glycogen in the SOL of TCS rats may be related to the increased glucose uptake in the presence of insulin. Accordingly the levels of glucose-6-phosphate are higher in fresh SOL of TCS rats and the ratio of glycogen synthetase_d to glycogen synthetase_i is also greater.

Carbohydrate metabolism has been shown to be affected in a number of ways by different models of hypokinesia. *In vivo* glycogen levels in the soleus muscle are known to be increased by short-term denervation (1) and harness suspension (2). In addition, exposure to 7 days of hypogravity also caused a dramatic increase in glycogen concentration in this muscle (3). We, therefore, sought to find the biochemical alterations caused by unloading that may bring about these increases in glycogen storage in the soleus.

METHODS

Female Sprague-Dawley rats were subjected to 6 days of tail-cast suspension (4). Muscles were excised rapidly, weighed, and either frozen in liquid nitrogen or used in incubation studies. In fresh-frozen tissue, glycogen (5,6), glycogen synthase activity (7), and glucose-6-phosphate levels (6) were assayed. Glucose uptake was determined by the uptake of 2-[³H]deoxy-D-glucose using [¹⁴C]insulin to correct for extracellular space (8). Isoproterenol (5 uM) was used in some incubation experiments to deplete the muscles of glycogen, in an attempt to reduce dilution effects

on metabolite measurements. Muscles were preincubated in isoproterenol/Krebs-Ringer bicarbonate buffer, washed in fresh buffer, and then incubated in buffer with and without insulin (10⁻⁴ U/ml). The muscles were assayed for glucose incorporation into glycogen and the media for lactate and pyruvate (6). Glucose oxidation was measured by ¹⁴CO₂ production from radiolabeled glucose (9). In a separate experiment, ¹⁴CO₂ production from radiolabeled pyruvate and pyruvate incorporation into glycogen were determined.

RESULTS AND DISCUSSION

In vivo Determinations. Following six-days of tail-cast suspension, the concentration of glycogen, the percent of total of glycogen synthase_d, and the level of glucose-6-phosphate were determined in fresh-frozen tissue. As shown in Table 1, there was a much greater glycogen concentration in the unloaded soleus muscle compared to the weight bearing control. In the unloaded soleus, the percentage of the glycogen synthase enzyme in the glucose-6-phosphate dependent form increased significantly. In this same group of muscles, the level of glucose-6-phosphate was much higher than in the control muscles. No changes were seen for any of these parameters in the extensor digitorum longus, a muscle known to be unresponsive to unloading (1).

These results suggest that the increased glycogen levels in the unloaded soleus may be due to an increased activity of the glycogen synthase_d as a result of higher glucose-6-phosphate levels.

In vitro Determinations. In an attempt to explain the above *in vivo* observations, a number of *in vitro* measurements were done. Dramatic changes in the responsiveness to insulin were found in the unloaded soleus. As presented in Table 2, the increase in 2-deoxy-glucose uptake due to insulin was much greater in the unloaded soleus as compared to the loaded muscle. Glucose incorporation into glycogen was also increased to a significantly greater extent by insulin in the unloaded soleus. When viewed together with the higher glucose-6-phosphate levels found *in vivo* this increased uptake of glucose due to insulin in the unloaded soleus could explain the increased glycogen levels found in this muscle. There was no differential effect of insulin on any of these parameters in the extensor digitorum longus.

In addition, glucose oxidation, and lactate and pyruvate release were also increased to a greater degree due to insulin in the unloaded soleus muscle (Table 2). However, pyruvate oxidation and pyruvate incorporation into glycogen (data not shown) were not differentially affected by insulin in either soleus or extensor digitorum longus muscles.

Concluding Remarks. From the data presented in this paper, a possible mechanism to explain the greater glycogen concentration in the unloaded soleus muscle can be described. The increase in the ratio of glycogen synthase_d to glycogen synthase_i together with an increased uptake of glucose due to insulin, which results in higher *in vivo* levels of glucose-6-phosphate, may cause an increase in glycogenesis. In the extensor digitorum longus, a muscle unresponsive to unloading, these changes are not seen, and the *in vivo* concentration of glycogen is the same in the loaded and unloaded muscles.

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Table 1. Changes in Fresh-frozen Suspended Soleus

Parameter	% Difference from Weight-Bearing
Glycogen concentration	+77 ^b
Glycogen synthase _d (% of total)	+23 ^a
Glucose-6-phosphate	+30 ^a

Significance of difference: ^aP<0.05; ^bP<0.001.

Table 2. Changes in Insulin Response in Suspended Soleus

Parameter	% Difference from Weight-Bearing
2-Deoxyglucose Uptake ^a	+ 74
Glucose Incorporation into Glycogen ^b	+125
Glucose Oxidation ^b	+ 26
Lactate and Pyruvate Release ^b	+417

^aDetermined at 10^{-1} U/ml insulin

^bDetermined at 10^{-4} U/ml insulin with glycogen depleted muscles.

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